varient residues, for example). The fact that all cytochrome c's have an isoelectric point near *p*H 9 is one manifestation of this compositional equilibrium, as is the fact that, for a given amino acid, the number of residues of that amino acid in any cytochrome c does not vary wildly from the mean value for the family. Specific sequence differences between proteins of the same family appear to be functional fine tuning resulting from the process of natural selection with respect to a given species (*8*). Similar statements hold for the hemoglobin chains, parvalbumins, lysozymes, and other protein families. There is no reason to believe that β-galactosidase is exempt from these general considerations (the preponderance of shorter proteins in previous compilations of protein structure is due to the fact that shorter proteins are easier to sequence).

The conclusion is that the observed amino acid composition of β-galactosidase from *E. coli* is not the sole result of a selectively neutral evolutionary process with respect to (i) base replacement at the gene level, (ii) equal interchangeability among amino acids, or (iii) replacement drawn from a pool of amino acids at their natural abundances in proteins.

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28 September 1978

Binding of *cis*- and *trans*-Dichlorodiammineplatinum(II) to DNA: Evidence for Unwinding and Shortening of the Double Helix

Abstract. *The antitumor drug* cis-*dichlorodiammineplatinum(II) (*cis-*DDP) and the inactive* trans *isomer bind and produce cooperative changes in closed and nicked circular duplex DNA's. Covalent binding of both platinum complexes to the closed circular DNA alters the degree of supercoiling, presumably by disrupting and unwinding the double helix. Electron micrographs show the platinated DNA's to be shortened by up to 50 percent of their original length. At similar ratios of bound platinum per nucleotide, the electrophoretic mobilities of the DNA's in gels containing the dye ethidium bromide are the same for both isomers. The only detectable difference in the binding of the two platinum isomers is an increase in the electrophoretic mobility in nondye gels of closed circular DNA having small amounts of bound* cis-*DDP that is not apparent for the* trans *complex.*

Cis-dichlorodiammineplatinum(II) (*cis*-DDP) is an antitumor drug of clinical importance (*1*, *2*). The *trans* isomer is inactive, however. Since DNA is widely believed to be the primary intracellular binding site of *cis*-DDP, many investigators have looked for differences in the DNA binding properties of the two platinum isomers (*3*, *4*). Previously we showed that *cis*-DDP binds covalently to closed circular PM-2 DNA under conditions (0.2*M* NaCl, 23°C) where *trans*-DDP does not (*5*). Here we report an extension of this work in which the solution conditions more nearly resembled those found intracellularly and in which the actual amount of platinum bound was measured. A remarkable change in the degree of supercoiling of the DNA was found by gel electrophoresis to accompany covalent binding of platinum. In addition, electron microscopy revealed a pronounced and progressive shortening of the DNA with increased platinum binding.

Closed circular DNA's used in this study were isolated from *Escherichia coli* strain K12 W677 cells containing the plasmid pSM1 (*6*). Closed circular, rather than linear, DNA's were employed because of the sensitivity with which small tertiary structural changes can be monitored (*7*) and because their superhelical nature better mimics that of

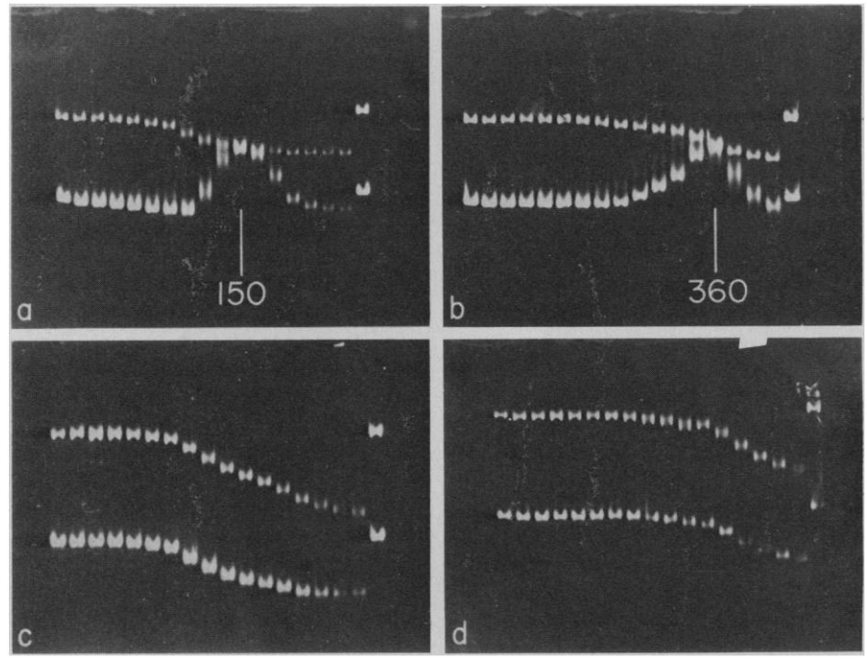

Fig. 1. Electrophoresis in 1 percent agarose gels of nicked and closed circular pSM1 DNA's incubated with (a and c) *cis*-DDP and (b and d) *trans*-DDP as a function of time. Gels contained 0.04*M* tris acetate, 0.02*M* sodium acetate, and 4 m*M* Na-EDTA, *p*H 8.1. The lower (c and d) dye gels also contained ethidium bromide (0.5 μg/ml). The upper (a and b) gels were stained with ethidium bromide (0.5 μg/ml) after electrophoresis. Within each slab, channels correspond to DNA samples (0.2 μg/ml) incubated with platinum reagent for (left to right) 0, 2.5, 5.5, 10, 15, 22, 30, 60, 90, 120, 150, 180, 240, 360, 480, 600, 720, and 0 minutes. Electrophoresis was conducted at 25 V for 15 hours at 4°C.

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certain forms of intracellular DNA, such as chromatin (*8, 9*). The *cis*- and *trans*-DDP were prepared by standard methods (*10*). The mole ratio of platinum bound per DNA phosphate (r) was determined by carbon arc atomic absorption spectroscopy. Samples were prepared for electron microscopy by the basic protein film procedure (*11*), stained with uranyl acetate, and shadowed with Pt-Pd.

In a typical experiment, a 250-μl portion of a mixture of closed and nicked circular DNA's at a total concentration of 390 μg/ml was dissolved in buffer containing 1 m*M* sodium phosphate, *p*H 7.4, 5 m*M* NaCl, and distilled water to a volume of 2.25 ml. The reaction was started by addition of 250 μl of a solution of *cis*- or *trans*-DDP, freshly prepared by dissolving the solid in distilled water. The final concentrations of *cis*- and *trans*-DDP were 87 and 43 μM, respectively, resulting in formal ratios (r_f) of Pt to DNA phosphate that ranged from 0.3 to 1.0. The reaction was terminated by adding NaCl [to retard the rate of formation of the reactive, solvolyzed forms of DDP (*12*)], to a final concentration of 0.2*M*, to portions removed from the reaction mixture at various times. Samples were frozen for subsequent analysis by gel electrophoresis, electron microscopy, and atomic absorption.

As shown in Fig. 1a, the electrophoretic mobilities of both the nicked and closed circular pSM1 DNA's change with incubation time (t_i). The time dependence reflects the kinetics of platinum binding, as independently verified by atomic absorption studies (see below). The increase in electrophoretic mobility of the nicked circular DNA with increasing amounts of bound platinum was surprising since attachment of the positively charged diammineplatinum(II) moiety to the anionic polymer would be expected to retard its migration in the gel. The platinum drug must therefore produce a more compact DNA structure on binding. Even more remarkable are the changes in the electrophoretic mobility of the closed circular DNA with platinum binding. For the data shown in Fig. 1a, the mobility proceeds through a slight maximum at 60 minutes, then diminishes at 150 minutes to a minimum coincident with that of the nicked DNA, then increases again relative to the mobility of the nicked DNA at yet longer times. These changes are reminiscent of those observed in progressive intercalation but, as shown previously (*5, 13*), *cis*- and *trans*-DDP do not bind DNA by an intercalative mode. The double helix must therefore be unwound by another

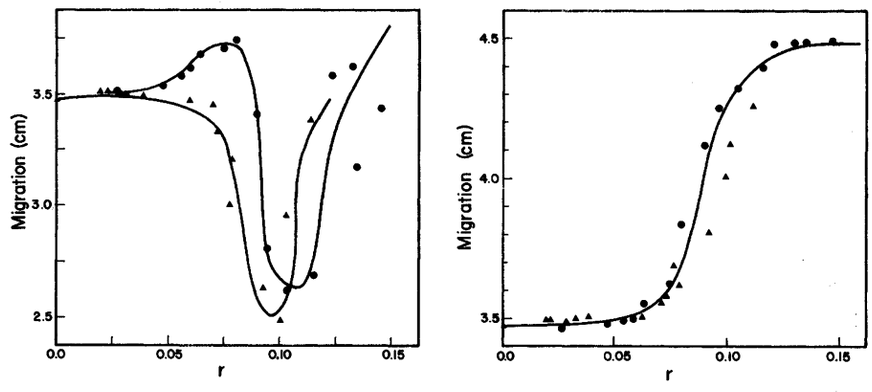

Fig. 2 (left). Migration of closed circular pSM1 DNA in 1 percent agarose gels as a function of the mole ratio of bound *cis*-DDP (•) and trans-DDP (▲) per nucleotide. Formal ratios (r_f) of added *cis*- and *trans*-DDP per nucleotide phosphate were 0.75 and 0.37, respectively. Distances of migration were measured from the gels shown in Fig. 1, a and b. Bound platinum concentrations were determined as follows: DNA (39 μg/ml) containing bound platinum reagent was separated from the incubation mixtures by spin dialysis (*14*) through Sephadex G-25-150 suspensions in 10 m*M* Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer, *p*H 7.4. The platinum-containing DNA samples were then analyzed with a Varian-375 atomic absorption spectrometer with a carbon rod atomizer. Fig. 3 (right). Migration of closed circular pSM1 DNA in 1 percent agarose dye gels (see text) as a function of the mole ratio of bound *cis*-DDP (•) and trans-DDP (▲) per nucleotide phosphate. Mobilities were measured from the gels shown in Fig. 1, c and d. Bound platinum concentrations were determined by atomic absorption as described in the legend of Fig. 2.

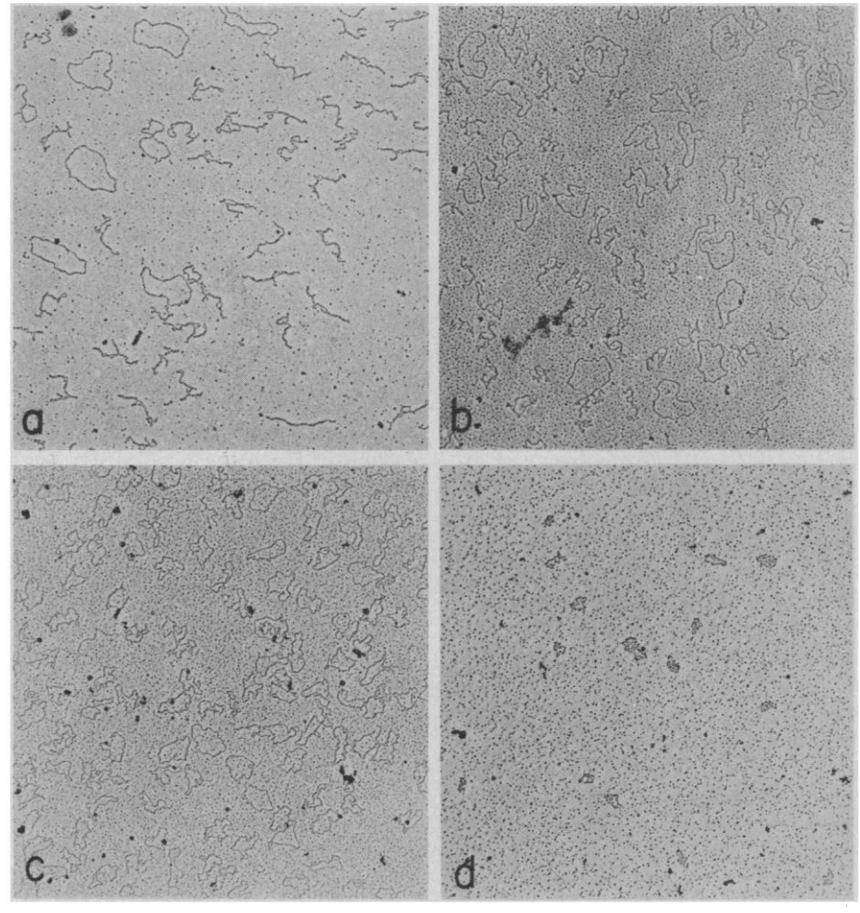

Fig. 4. Electron micrographs of pSM1 DNA incubated with *cis*-DDP. DNA samples were incubated with platinum reagent for (a) 0, (b) 60, (c) 180, and (d) 360 minutes, corresponding to r values of 0, 0.075, 0.105, and 0.135, respectively. Micrographs were recorded by using a Phillips-EM201 electron microscope with samples prepared as described in (*11*).

process brought about by covalent binding of platinum. We suggest that drug binding disrupts the hydrogen bond formation and causes localized unwinding of the duplex. The interruption of base pairing would produce single-stranded regions that, under the conditions of low ionic strength in the gels, would collapse and reduce the effective length of the DNA's.

Although comparison of Fig. 1, a and b, would suggest differences in the effects of *cis*- and *trans*-DDP on the electrophoretic mobilities of the circular DNA's, plots of gel migration against bound platinum per nucleotide (r) (Fig. 2) show otherwise. The minima in these plots ($r \sim 0.10$) are nearly coincident for both isomers. One notable difference, however, is the increase in mobility observed for *cis*-DDP at $r < 0.075$. This reproducible difference in gel mobilities at low r values is not yet understood. It is interesting that the characteristic increase in mobility at low r exhibited by the DNA-*cis*-DDP complex resembles that observed when ethidium bromide binds by intercalation to closed circular DNA.

The effects of *cis*- and *trans*-DDP on the electrophoretic mobilities of the closed circular DNA's are also manifest in gels containing ethidium bromide (*5*) (Fig. 1, c and d). The presence of this intercalator in the gel ensures that the closed circular DNA is wound into a net positive superhelix and suppresses the effect on electrophoretic mobility of changes in the superhelix density in regions of unbound platinum. Figure 3 is a plot of migration against r for these dye gels, from which it is evident that both *cis*- and *trans*-DDP produce cooperative changes in the mobility of the DNA's. The r value at which the cooperative effect occurs is identical with that of the mobility minimum observed when either isomer binds to the closed DNA (Fig. 2). The increased mobilities (Fig. 3) again indicate a partially collapsed structure.

The electrophoretic mobility results suggest that the binding of DDP shortens the length of the DNA. This interpretation has been directly confirmed by electron microscopy. As shown in Fig. 4 for the *cis* isomer, the DNA molecules become more compact with increasing r. The opening and subsequent superhelical twisting of the closed circular DNA is clearly evident. Histograms (not shown) of length measurements show the shortening to be as much as 50 percent of the original DNA size. The precise nature of the platinum-DNA interaction that produces this interesting collapse of the double helix is not known. The binding must be covalent, however, since ionic or hydrogen bonding interactions would be reversed under the high chloride concentrations used to quench the reaction. Weakly bound platinum would also separate from DNA in gel electrophoresis.

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21 September 1978; revised 18 December 1978

Regional Localization of the Gene for Human Phosphoribosylpyrophosphate Synthetase on the X Chromosome

Abstract. *Sixty-eight independent hybrid clones were isolated after irradiated normal human lymphocytes were fused with Chinese hamster fibroblasts lacking hypoxanthine-guanine phosphoribosyltransferase activity. The cells were grown under selective conditions requiring retention of the X chromosome–linked locus for human hypoxanthine-guanine phosphoribosyltransferase. The frequency and patterns of cotransference of human phosphoribosylpyrophosphate synthetase with the selected marker and with additional X-linked enzymatic markers confirm X linkage of the structural gene for human phosphoribosylpyrophosphate synthetase and support assignment of this gene to a position on the long arm of the X, between the loci for α-galactosidase and hypoxanthine-guanine phosphoribosyltransferase.*

5 - Phosphoribosyl - 1 - pyrophosphate (PRPP) is an activated sugar phosphate utilized in the synthesis of purine, pyrimidine, and pyridine nucleotides. PRPP is a substrate common to the initial reaction of the pathway of purine synthesis de novo, catalyzed by amidophosphoribosyltransferase (E.C. 2.4.2.14), and to purine base salvage reactions catalyzed by hypoxanthine-guanine phosphoribosyltransferase (HGPRT; E.C. 2.4.2.8) and adenine phosphoribosyltransferase (E.C. 2.4.2.7). That PRPP availability has a role in the regulation of the rate of purine synthesis de novo is suggested by the observations that (i) PRPP is a limiting substrate and an allosteric activator of amidophosphoribosyltransferase (*1*), the presumed rate-limiting enzyme of the pathway, and (ii) increased concentrations of PRPP are found in cells derived from individuals with increased purine nucleotide production caused by either excessive PRPP synthetase (E.C. 2.7.6.1) activity (*2*) or deficiencies (severe or partial) of HGPRT (*3*).

The enzyme PRPP synthetase catalyzes the synthesis of PRPP from adenosine triphosphate (ATP) and ribose-5-phosphate in a complex reaction requiring inorganic phosphate and Mg^{2+}. Human erythrocyte PRPP synthetase consists of a single subunit species capable of reversible self-association to a variety of aggregated forms in a process mediated by effectors of enzyme activity and by enzyme concentration (*4*). Several families have been identified in which structurally altered forms of PRPP synthetase with increased enzyme activity result in increased PRPP production, excessive purine nucleotide and uric acid synthesis, and clinical gout (*2*). Fibroblast cultures from heterozygous female members of two of these families have been shown, by direct and indirect means (*5*), to contain two clones of cells expressing distinct phenotypes with respect to PRPP synthetase. This finding is in accord with the specific chimerism predicted for females heterozygous for X-linked traits by the Lyon hypothesis (*6*) of random X-chromosome inactivation and

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